DiI cell labeling in lamprey embryos.

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Public Summary:
Here we describe a method for in vitro fertilization of lamprey embryos and how to culture them to desired embryonic stages injecting reagents (e.g. inhibitors) into early lamprey embryos in order to perturb aspects of their development and examine the subsequent changes.

Scientific Abstract:
Lampreys are one of the most basal animals in which many of the true vertebrate characteristics (e.g., neural crest, placodes, segmented brain, skull, paired sensory organs, pharyngeal skeleton) are present. Studying the molecular and developmental mechanisms responsible for the formation of these structures in lamprey and higher vertebrates can provide insight into how these vertebrate characteristics evolved. The relative ease of obtaining mature adults and embryos makes this animal an ideal model for investigations into early vertebrate evolution. In addition, studies of features that are unique to lampreys can provide insights into mechanisms of parallel evolution. Lamprey embryos are particularly amenable to injection techniques. Like zebrafish and Xenopus embryos, they have double chorions and are resistant to surface-tension-induced rupture when removed from liquid. They can therefore be injected in a dry dish; this eliminates the need to support the embryo while performing injections and makes the procedure very rapid. Also, a single ovulating female can contain up to 100,000 eggs, so the number of injectable embryos per fertilization is not a limiting factor. This protocol describes how to label lamprey embryo cells by microinjecting the fluorescent dye DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) to study cell fate during development.